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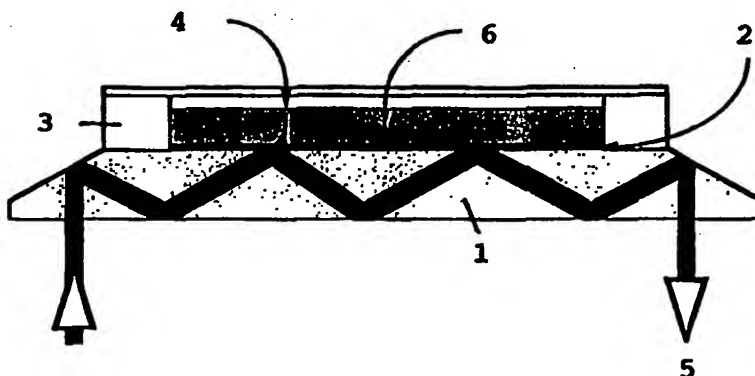
(51) International Patent Classification ⁶ : G01N 21/55		A1	(11) International Publication Number: WO 99/05509
			(43) International Publication Date: 4 February 1999 (04.02.99)
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Published
With international search report.

(54) Title: **DETECTION AND INVESTIGATION OF BIOLOGICAL MOLECULES BY FOURIER TRANSFORM INFRA-RED SPECTROSCOPY**

(57) Abstract

Method and apparatus to study biological molecules or biological components at or in self-assembled monolayers (SAMs) on metal surfaces (2) such as gold in aqueous environments (6) using attenuated total internal reflection Fourier transform infra-red (ATR-FTIR) spectroscopy are described. Said method may be used for e.g. the screening for effective substances suitable as agonists or antagonists or - if performed time resolved - for studying kinetics of interactions of e.g. biological molecules adsorption and desorption processes or for the development of materials and surfaces for biological or medical applications.



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Detection and Investigation of Biological
Molecules by Fourier Transform Infra-red Spectroscopy

5 Technical field

 The invention concerns methods and devices
for the detection and investigation of biological mole-
cules at or in self-assembled monolayers on metal sur-
10 faces using infrared spectroscopy in the attenuated total
internal reflection configuration.

 Background Art

15 In recent years, self-assembled monolayers
(SAMs) have been intensively studied because of their
relevance to both science and technology (Ulman, A. *An
introduction to ultrathin organic films: from Langmuir-
20 Blodgett to self-assembly*; Academic Press: San Diego,
1991). SAMs are monomolecular layers formed on solid sur-
faces by the immersion of a substrate into a solution of
the appropriate surfactant. The surfactant contains a
functional group which reacts specifically with the sur-
25 face: chemisorption of the surfactant to the surface pro-
duces a highly ordered densely packed monolayer. The high
stability and reproducibility of SAMs, the wide range of
surface functionalities that can be produced, and their
ease of manufacture makes them potentially applicable in
30 many fields including corrosion prevention, wear protec-
tion, adhesion, electrode surfaces, photoactive materi-
als, biocompatibility, biosensing, etc. Various surfac-
tant/substrate pairs have been used for the formation of
self-assembled monolayers. However the most extensively
35 characterized and technologically relevant of all SAMs
are those of sulfur-containing molecules on metals (e.g.
Au, Ag, Cu, Pt). SAMs offer a unique opportunity in sur-

face science since they allow the easy creation of well-defined surfaces whose properties can be systematically varied.

Of particular interest is the application of SAMs to biological systems. Numerous studies using gold surfaces modified with thiols or disulfides have investigated such processes as: the non-specific adsorption of proteins at surfaces (biocompatibility); the formation of supported lipid layers and the incorporation of biological molecules in these layers; and the immobilisation of proteins via molecular recognition. These studies have demonstrated the great potential of SAMs for the investigation, characterisation and detection of biological molecules and their interactions with surfaces, with each other and with other species in solution. For such studies, non-destructive *in situ* techniques applicable in aqueous media are essential.

Unfortunately, few such techniques are available for the study of these processes *in situ* on metal surfaces. The study of ultra-thin organic films on solid substrates constitutes a considerable experimental challenge because of the extremely small quantity of material present in the film. Thus, extremely sensitive or highly surface specific techniques are necessary, to extract the small signal of the organic film from the high background of the surroundings. A wide range of appropriate techniques are available under vacuum conditions. However, the number of techniques that can be applied in water, is quite limited.

For ultra-thin organic films on metal surfaces, the number of techniques available is reduced even further. This is due firstly, to the opacity of the metal substrate and secondly, and very importantly, to the fact that metal substrates quench fluorescence. The most commonly used analytical techniques for metal surfaces in aqueous media are: surface plasmon resonance (which measures mass loading of the surface); scanning probe micro-

scopies; electrochemical techniques such as ac impedance spectroscopy and cyclic voltammetry; neutron scattering. These techniques have very limited access to structural information about chemical and biological species at the surface - molecular packing, orientation, chemical structure - especially if time-resolved information is required.

Fourier transform infra-red (FTIR) spectroscopy is an extremely powerful analytical technique which can be used to identify, quantify and obtain information about the molecular structure, orientation and ordering of organic species (Griffiths, P. R.; de Haseth, J. A. *Fourier Transform Infrared Spectroscopy*; John Wiley: New York, 1986; Vol. 83). It is particularly relevant for biological systems since detailed analysis of, for example, the amide peaks gives a great deal of information about protein conformation. Similarly, the CH₂ bands of lipids can be used to study lipid membrane fluidity - an extremely important parameter in cell membrane processes. The use of infrared spectroscopy for the investigation of monolayer and multilayer organic samples in air or vacuum is well-developed both for oxide surfaces (attenuated total internal reflection configuration, ATR) and for metal surfaces (grazing incident angle configuration). However the analysis of ultra-thin organic films at metal/water interfaces remains very difficult. This is due to the very high absorption of infrared light by water, which renders the grazing incident angle configuration unworkable. Most studies of metal/ water interfaces use a modified grazing incident angle configuration with the thinnest possible water cell (1-10 μ m thickness, formed by clamping the metal surface against the cell window), and a much higher angle of incidence (Faguy, P. W.; Marinkovic, N. S. *Anal. Chem.* 1995, 67, 2791). This configuration is inappropriate for studies of biological processes at self-assembled monolayers because the study of binding and release reactions at SAMs requires addition of sub-

stances to the surface, which is only possible on removing the metal surface from the IR window. This has two disadvantages: firstly, kinetics measurements of these reactions are made very difficult; secondly, repeated pressing of the metal surface against the IR window may damage the SAM, thus inducing artifacts.

The use of thin gold films (5-10nm thick) as substrates for self-assembled monolayers has been described by DiMilla et al (DiMilla, P. A.; Folkers, J. P.; Biebuyck, H. A.; Härter, R.; Lopez, G. P.; Whitesides, G. M. *J. Am. Chem. Soc.* 1994, 116, 2225) who demonstrated that the SAMs formed on these substrates are comparable if not superior to those formed on bulk gold substrates and can be investigated using UV/vis spectroscopy.

Infrared approaches have been described for combined infrared and electrochemical studies at metal surfaces. However, in none of these cases have self-assembled monolayers been used to control the properties of the metal/water interface as would be necessary for detailed investigations of biological systems.

Thus, there is still a need for an infra-red method allowing the study of biological molecules in such environments.

Object of the Invention

The present invention provides a method to study biological molecules at or in self-assembled monolayers on metal surfaces, particularly gold surfaces, in aqueous environments by using attenuated total internal reflection (ATR) infra-red (IR) spectroscopy, preferably, because of its improved sensitivity, Fourier transform infra-red (FTIR) spectroscopy. This technique is based on the use of thin films (1 to 30 nm, preferably 3 to 15 nm, particularly preferred 5 to 10 nm) of gold or other metals deposited on ATR elements.

The inventive method allows the study of biological molecules which form a self-assembled monolayer (SAM) and/or biological molecules which are immobilised on a SAM and/or biological molecules which react with a SAM, their interactions with surfaces such as cells or cell fragments or their interactions with other biological molecules, with ions or with other water-soluble molecules. Such ions and water-soluble molecules are understood as also comprising complexes.

In the inventive application, the relative transparency of the thin metal films, e.g. gold films, in the infra-red is exploited: internal reflection of the infra-red beam at the interface between the ATR element and the metal produces an evanescent field which penetrates through the metal film and into the aqueous phase on the other side. This allows sampling of SAMs and of biological molecules at or in the SAMs at the metal/water interface. Multiple reflections may be used to increase the intensity of the infrared spectrum.

It is thus an object of the invention to provide a simple method for the infrared investigation of biomolecules in aqueous media at or in self-assembled monolayers at surfaces of gold or other metals, their binding and release reactions at or on these surfaces, and the associated changes in their conformation and structure.

It is a further object to provide this information time-resolved for the study of the kinetics of these processes, particularly with a high time resolution, preferably on the seconds scale.

It is still a further object to provide a device for such studies and a method for producing such a device.

Brief Description of Drawings

Figure 1 is a schematic cross-section of an inventive device,

Figure 2 is a comparison of spectra of a N^{α}, N^{α} -bis(carboxymethyl)- N^{ω} -(11-mercaptoundecanoyl-glycyl-glycyl-glycyl)-L-lysine (CTA) layer and N^{α}, N^{α} -bis(carboxymethyl)-L-lysine (lysine-NTA) in solution without (part A) and with (part B) ion (Ni^{2+}) complexation,

Figure 3 is a difference spectrum between a nickel-charged CTA SAM with Fab fragment and the same SAM without Fab,

Figure 4 shows the adsorption kinetic of the Fab fragment on the CTA SAM.

Methods for carrying out the invention

The present invention concerns a method to study biological molecules or their interaction or complexation or reaction with ions and/or biological molecules and/or biological components and/or other molecules at or in self-assembled monolayers (SAMs) on gold or other metal surfaces in aqueous environments by using attenuated total internal reflection (ATR) infra-red (IR) spectroscopy, particularly Fourier transform infra-red (FTIR) spectroscopy. This technique is based on the use of thin films (about 1 to about 30 nm) of metals, particularly gold, deposited on ATR elements. Preferably such metal films have a thickness of 3 to 15 nm, particularly 5 to 10 nm. Metals other than gold are e.g. Ag, Cu, Pt, Au/Pt, alloys, particularly gold comprising alloys, multilayers, particularly bilayers of metals such as gold on chromium or titanium. The deposition of e.g. gold on a very thin layer of another metal is suitable for the formulation of thin gold films with smooth surfaces.

The self-assembled monolayer (SAM) is prepared by contacting a first solution comprising a self-assembled-monolayer-forming molecule to at least one metal-coated surface of said attenuated total internal reflection (ATR) element.

Said SAM may be further reacted with other molecules to give new surface functional groups or to form one or more additional layer(s) on the metal surface, thus enabling the study of said new surface functional groups or said one or more additional layer(s) at the SAM, their interaction or complexation or reaction with ions and/or biological molecules and/or biological components and/or other molecules in solution at the SAM.

If interactions of the SAM are the subject to be studied, then the first solution of the self-assembled-monolayer-forming molecule can be removed and a second or further solution comprising a metal ion and/or a biological molecule and/or other molecules can be applied to the monolayer in order to study the interactions of self-assembled monolayers with ions and/or biological molecules and/or other molecules, and/or cells or cell fragments, with at least those solutions being in contact with biological molecules and/or components being aqueous solutions. If the SAM comprises itself biological molecules, then the molecules of the second or further solution usually of course will be different from those of the SAM.

The present invention also concerns a method to provide such information time-resolved for the study of the kinetics of such processes, particularly for studies with a good time resolution, preferably a time resolution on the seconds scale.

The method of the present invention is e.g. a suitable method for a screening for effector compounds and for the evaluation of the activity of pharmacological agents as agonists or antagonists for biosensitive receptor proteins. Such screening becomes more and more important for the detection of substances that are e.g. active modulators of membrane proteins.

The inventive method provides a sensitive method for an efficient drug screening of e.g. such modulators with high throughput.

The study of molecules or components of interest may include their identification or quantification, or their functional characterization or characterization of their conformation, orientation or ordering using or based on their specific infra-red spectra.

The present invention furthermore concerns a solid device as e.g. the one shown in Figure 1. Said solid device (ATR element) 1 is transparent in the infra-red and one or more surfaces thereof are coated with a thin film 2 of gold or of another suitable metal or alloy or mixture of metals as described above.

The coating with a metal layer is such as to enable generation of an evanescent field 4 at the interface water/metal, said metal layer 2 being transparent to an infra-red beam 5.

Said thin metal layer 2, for the use in the inventive method must be provided with a SAM.

As already outlined above, several metals, alloys or multilayers are suitable ATR coatings, presently, however, gold, gold alloys and bilayers with gold surface are preferred. Preferred ATR elements are made from a material chosen from the following group: germanium, silicon, ZnSe, ZnS, AMTIR (an amorphous glass of germanium, selenium and arsenic).

The thin metal layer has a thickness from about 1 to about 30 nm, preferably 3 to 15 nm, particularly 5 to 10 nm. The self-assembled monolayer is formed of molecules containing metal-surface reactive groups, preferably chosen from the following groups: thiols, disulfides, thiophenes, phosphines and isonitriles.

Such an inventive device can be produced by coating an attenuated total internal reflection (ATR) element with a thin metal layer on at least one face. Such coating is performed by means of known methods for the preparation of thin metallic films, e.g. physical vapour deposition (PVD).

For being used according to the inventive method, said at least one metal coated face of the inventive device (ATR element) is then pressed against a partial cell (said partial cell provides the further cell walls) thus that a water-tight cell 3 is generated of which at least one face or part of one of the faces is formed by a metal layer of the ATR element.

Such a cell consisting of an inventive device and a partial cell thus combined that they form a cell 3 with one face being at least partially formed by the ATR metal coating is also part of the present invention. Such a cell can have varying volumes, preferably volumes easily allowing the replacement of the solution comprised therein.

According to the inventive method the metal-coated surface or surfaces are first brought into contact with a solution (not necessarily an aqueous solution) comprising molecules suitable to form a self-assembled monolayer. Such molecules may be biological molecules to be studied or an other molecule the interaction of which with biological molecules shall be studied or which shall be used for the immobilisation of biological molecules on the surface. For the formation of a suitable surface further reactions of the SAM or further self-assembly steps may be necessary, to give suitable functional groups or a suitable environment for the biological molecules to be studied. For the study of complexations and interactions the first solution is removed and replaced by another solution comprising the interacting biological molecule and/or biological component and/or ion and/or other molecules present in the solution. Since the self-assembled monolayer and/or the interacting molecule are biological molecules or biological components such as cells, cell fragments (e.g. parts of the cell membrane), at least the second and/or further solutions are aqueous solutions. The properties (e.g. hydrophilicity, protein binding properties, ion complexation abilities) of the metal

coated surface or surfaces are defined by the self-assembled monolayer which is formed on the metal surface: the choice of the appropriate molecules or mixture of molecules for the SAM allows control of these surface properties. As already mentioned above the SAM may be formed of biomolecules (e.g. peptides, DNA, RNA, sugars) or may be formed of other suitable molecules (e.g. alkanethiols, hydroxyalkanethiols, carboxyalkanethiols). The SAM may be formed on the metal-coated surface either before or after bringing it into contact with an aqueous medium.

The inventive method generally allows the investigation of biological molecules, for example, proteins (such as receptors, enzymes, antibodies, transmembrane proteins), DNA, RNA, polysaccharides, lipids, natural or synthetic peptides. Biological components may also be investigated: for example whole cells or cell fragments.

An infrared beam incident on the device passes inside it where it is internally reflected at one or more of the metal-coated surfaces before exiting the device for spectroscopic analysis. The thickness of the metal coating is chosen so that internal reflection of the infrared beam in the device produces an evanescent field at the SAM at the interface between metal and aqueous medium. It has been found that the interactions of said evanescent field with the biomolecules present at or in the SAM are observable in the spectrum of the infrared beam which exits the device and thus allows their investigation and study.

As already mentioned above, for IR measurements in the ATR configuration, the optimal film, e.g. gold film, must be thin enough that the evanescent field at the metal, particularly gold, water interface has a high intensity, while the reflectivity of the metal/ATR element interface must also be relatively high. This optimal film thickness is a function of the optical parame-

ters chosen e.g. detector sensitivity, number of reflections at the metal coated surface. For optimal SAMs, the film must adhere well to the ATR element and form a continuous smooth metal layer covering the entire surface of the ATR element. Scanning electron microscopy and conductivity measurements of gold layers indicate, however, that even films with defects such as holes comprising as much as about 15-20 % of the surface are already suitable for some investigations.

10 A specific inventive device as represented in Figure 1, suitable for the inventive method can be produced and used as follows.

A germanium attenuated total internal reflection (ATR) element 1 is coated with a thin layer 2 (approx. 3nm) of gold on one face. This face is pressed against a cell to form a water-tight seal. The cell may be of any inert material, however, the material preferably is polytetrafluoroethylene (herein further on referred to under its generally used trade mark name Teflon®). The gold film is used as a substrate for self-assembly of e.g. thiol monolayers. Evanescent waves 4 are produced upon application of an infra-red beam 5. The cell is filled with aqueous solution 6. When the spectral region of interest is between 1800-1400cm⁻¹, heavy water (D₂O) media may be used. Such a device (with D₂O medium) is used in the following examples.

Other substances transparent in the infrared, and other metal films or film thicknesses may also be used (see above).

30 It has been found that not only a specific SAM can be used more than once, but that the SAM on either the metal surface or the precursor-treated metal surface can be replaced if need be and even the metal layer can be removed by well established chemical methods without affecting the ATR-element and a new metal layer can be applied to the ATR-element, thus allowing its multiple use.

Since the present method is particularly suitable for investigations with small amounts of interesting material, it is of course much preferred to use the more sensitive Fourier transform infra-red spectroscopy than usual dispersive IR spectroscopy.

The following examples serve as an illustration of the invention, but should not be construed as limitation thereof.

Example 1: Investigation and characterisation of binding reactions of a peptide self-assembled monolayer with such a device.

Step 1: Preparation of the device

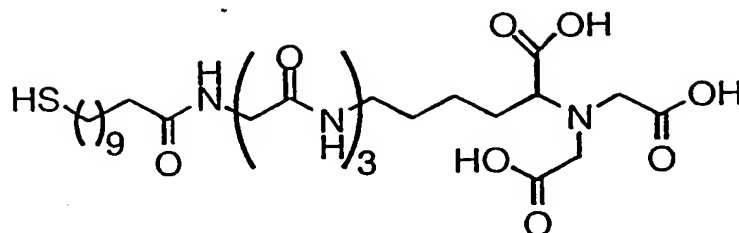
A device similar to that shown in Figure 1 and described above is prepared by thermal evaporation in vacuum of less than 10^{-5} mbar of a thin layer of gold onto one surface of a germanium ATR element at room temperature (60x10x4mm with an internal angle of incidence of 45°). The ATR element was prepared for this treatment by silanisation with 3-(mercaptopropyl)trimethoxysilane, which is a suitable treatment for the formation of thin gold films with smooth surfaces.

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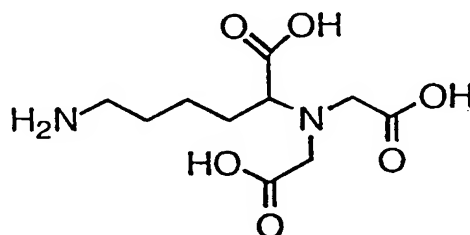
Step 2: Formation of a SAM of a peptide on the device

After coating with gold, the gold surface was exposed to a solution of the peptide N^α, N^α -bis(carboxymethyl)- N^ω -(11-mercaptoundecanoyl-glycyl-glycyl-glycyl)-L-lysine ("chelator thioalkane" or CTA) in heavy water for two hours and was then washed with heavy water. CTA has the following formula

35



This lead to the formation of a self-assembled monolayer (SAM) of CTA on the gold surface. The molecule N^{α}, N^{α} -bis(carboxymethyl)-L-lysine (lysine-NTA) was used in solution in transmission IR measurements, for comparison. Lysine-NTA has the following formula:



Step 3: Analysis of the peptide SAM

Spectra of the CTA SAM were obtained by comparing spectra of the device after treatment with CTA with spectra of the device before. Spectra of CTA monolayers and the bare gold surface were recorded in deuterated buffer (20mM sodium phosphate, 250mM NaCl, pD 7.5). These spectra are shown in Figure 2, Part A and compared with transmission spectra of a thin film of aqueous solution (~60µm) of the comparison molecule lysine NTA taken in the same buffer.

The lysine-NTA spectrum has two major peaks at 1625cm^{-1} and at 1402cm^{-1} . These are assigned to the carboxylate asymmetric (ν_{asym}) and symmetric stretch (ν_{sym}) respectively. (The peak at 1730cm^{-1} is assigned to a minor component of protonated carboxylic acid groups.) The spectrum of the CTA SAM at the gold surface retains

the two major peaks: the peak at 1624cm^{-1} is attributed to the carboxylate asymmetric stretch and the amide I' band of the 4 peptide bonds in the CTA spacer; the peak at 1403cm^{-1} is attributed to the carboxylate symmetric stretch; while the new peak at around 1467cm^{-1} is attributed to the amide II' bands of the (deuterated) peptide bonds with a contribution from the $\delta(\text{CH}_2)$ modes.

Step 4: Investigations of the ion complexation reactions of the peptide SAM

The ion complexation reactions of the peptide SAM were studied by incubating the CTA monolayer with a solution containing Ni^{2+} ions in heavy water (50mM Ni_2SO_4) for one minute. After washing, spectra were recorded in deuterated buffer (20mM sodium phosphate, 250mM NaCl, pD 7.5). The spectra of the CTA monolayer were compared with transmission spectra of lysine-NTA recorded in 20mM sodium phosphate, 250mM NaCl, 20mM NiSO_4 , pD 7.5 as shown in Figure 2, Part B.

Large changes in the spectra may be observed: in the lysine-NTA spectrum a new peak appears at 1592cm^{-1} and is attributed to the asymmetric stretch of the nickel-complexed carboxylate group. The uncomplexed asymmetric and the symmetric carboxylate peaks are shifted slightly to 1628cm^{-1} and to 1410cm^{-1} , respectively. Similarly, for the CTA SAM, a new peak appears in the spectrum at around 1591cm^{-1} . The three peaks present in the previous CTA SAM spectrum (without Ni^{2+}) are now found at 1625cm^{-1} , 1472cm^{-1} , and 1406cm^{-1} .

Thus we are able to observe the characteristic carboxylate and peptide peaks of CTA in the SAM. The spectra observed correspond very well with transmission spectra of the headgroup, and with literature spectra of NTA in solution. Complexation of Ni^{2+} by the peptide SAM can clearly be observed.

Example 2: Investigation of the binding reactions of a Fab fragment with such a device.

5 Step 1: Preparation of the device

A device was prepared as described in example 1, a self-assembled monolayer of CTA was formed on the gold surface, and the CTA SAM was incubated with nickel ions and washed.

10

Step 2: Binding of Fab fragment to the device

The anti-lysozyme Fab fragment D1.3 bearing a hexahistidine extension at the C-terminus of the heavy
15 chain was equilibrated in deuterated buffer for a period of several days. The absence of an amide II peak in the spectral region $1510\text{--}1580\text{cm}^{-1}$ was indicative of quasi-complete exchange of amide protons for deuterons.

The metal-coated surface of the device, with
20 the nickel-charged CTA SAM was incubated with a $1\mu\text{M}$ solution of the Fab in deuterated buffer (20mM sodium phosphate, 250mM NaCl, pD 7.5) for 30 minutes. After washing with buffer, infrared spectra were recorded. The spectrum shown in Figure 3 is the difference spectrum between a
25 nickel-charged CTA SAM with Fab and the same SAM without Fab. The frequency range $1700\text{--}1600\text{cm}^{-1}$ is shown as this is the range in which the amide I peak is found. The amide I peak is characteristic of the secondary structure of the protein.

30 Transmission spectra of the Fab in solution were also collected using a $6\mu\text{M}$ solution in deuterated buffer and were compared with the spectra collected using the device as shown in Figure 3. Both spectra have a broad triangular peak with a maximum at 1636cm^{-1} characteristic of the β -sheet conformation of Fab fragments. A
35 small difference in the intensities centered around 1660cm^{-1} can be observed, but in general the two spectra

are almost identical. Thus this device allows us to obtain good protein IR spectra with protein solutions of relatively low concentration: the solution used for the ATR spectrum contained 0.05mg/ml protein, compared to the
5 10-30mg/ml normally used for transmission IR spectra of proteins. The amide I peak gives us a great deal of information about the secondary structure of the Fab fragment (in this case β -sheet) and demonstrates that the protein undergoes no significant changes in secondary
10 structure on binding to the CTA SAM.

Step 3: Desorption of Fab fragment from the device

15 After adsorption of the Fab fragment, the device was incubated with a deuterated solution of imidazole (250mM imidazole in 20mM sodium phosphate, 250mM NaCl, pD 9.5) for 10 minutes and washed with buffer. New spectra of the Fab revealed that 80-90% of the protein
20 was desorbed from the gold surface.

Example 3: Time resolved measurements of the binding reactions of the Fab to the device.

25 Step 1: Preparation of the device

The device was prepared with a nickel-charged CTA SAM as described in examples 1 and 2.

30 Step 2: Measurement of the adsorption of the Fab

The device was incubated with a deuterated solution of Fab fragment as described in example 2. Before addition of the solution of Fab, initial spectra of the device surface were measured. Numerous spectra were
35 measured at regular intervals during the entire adsorption process until it had finished. The intensities of the amide I peak for the different spectra were measured

and the integrated intensity plotted against time as shown in Figure 4 to obtain the adsorption kinetic for the Fab fragment.

Patent Claims

5 1. A method for the study of biological molecules and biological components or their interaction or complexation or reaction with ions and/or biological molecules and/or biological components and/or other molecules in solution at or in self-assembled monolayers on
10 surfaces of thin metal layers in aqueous environment wherein attenuated total internal reflection (ATR) infrared (IR) spectroscopy is applied on self-assembled monolayers prepared by contacting a first solution comprising a self-assembled monolayer forming molecule to at least
15 one face of said ATR element coated with said metal layer.

 2. The method of claim 1 wherein the metal is gold, a gold comprising alloy or gold comprising bilayer.

 3. The method of claim 1 or 2 wherein the
20 metal layer has a thickness of about 1 to about 30 nm, preferably 3 to 15 nm, particularly 5 to 10 nm.

 4. The method of any one of claims 1 to 3 wherein the first solution of the self-assembled monolayer forming molecule is removed and wherein a second or
25 further aqueous solution comprising a metal ion and/or a biological molecule and/or a biological component and/or other water-soluble molecule is applied to the monolayer in order to study the interaction or complexation or reaction of self-assembled monolayers with ions and/or biological molecules and/or biological components and/or
30 other water-soluble molecules in solution.

 5. The method of claim 4 wherein the second aqueous solution comprises metal ions or molecules other than biological molecules.

35 6. The method of claim 4 or 5 wherein the second or further aqueous solution comprises biological molecules, said molecules being different from the mole-

cules of the monolayer in order to study interactions thereof with the self-assembled monolayer.

7. The method of any of claims 1 to 6 which is performed time-resolved.

5 8. The method of claim 7 which is used for kinetic studies.

9. A device suitable for the investigation of biological molecules and biological components and their interaction with surfaces or with ions or with molecules
10 in solution, consisting of an attenuated total internal reflection element (1), transparent in the infra-red, coated on at least one face with a thin metal layer (2) and with a self-assembled monolayer (SAM).

10. A device according to claim 9, wherein
15 the metal layer (2) is gold or an alloy containing gold or a bilayer containing gold.

11. A device according to claim 9 or 10, wherein the attenuated total internal reflection element (1) is made from a material chosen from the group of materials consisting of germanium, silicon, ZnSe, ZnS, AM-
20 TIR.

12. A device according to any one of claims 9 to 11, wherein the thin metal layer (2) has a thickness from about 1 to about 30 nm, preferably from 3 to 15 nm,
25 particularly from 5 to 10 nm.

13. A device according to any one of claims 9 to 12, wherein the self-assembled monolayer is formed of molecules containing metal surface reactive groups chosen from the following groups: thiols, disulfides, thio-
30 phenes, phosphines, isonitriles.

14. A device according to any one of claims 9 to 13, which furthermore comprises a partial cell combined with the self-assembled monolayer carrying metal surface (2) of the attenuated total internal reflection
35 element (1) thus that the self-assembled monolayer carrying metal surface (2) of the attenuated total internal

reflection element (1) forms at least a part of at least one face of a water-tight cell (3).

15 15. A method for producing a device according to any one of claims 9 to 13 wherein an attenuated total internal reflection (ATR) element is coated with a thin metal layer on at least one face, said at least one face then being treated with a self-assembled monolayer (SAM) forming molecule.

10 16. The method of claim 15, wherein said at least one face of the attenuated total internal reflection element coated with a thin metal film is pressed against a partial cell, thus that a cell (3) is generated, at least one of the faces of which is said metal layer (2), said cell being generated prior or after ap-
15 plying a SAM to said metal layer (2).

17. The use of a device according to any one of claims 9 to 14 for studying biological molecules or biological components or their interaction or complexa-
20 tion or reaction with metal ions or biological molecules or biological components or water-soluble molecules at or in self-assembled monolayers.

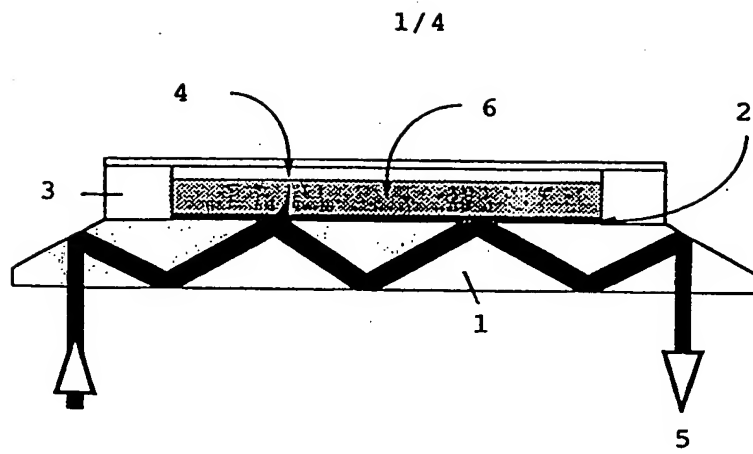


Figure 1

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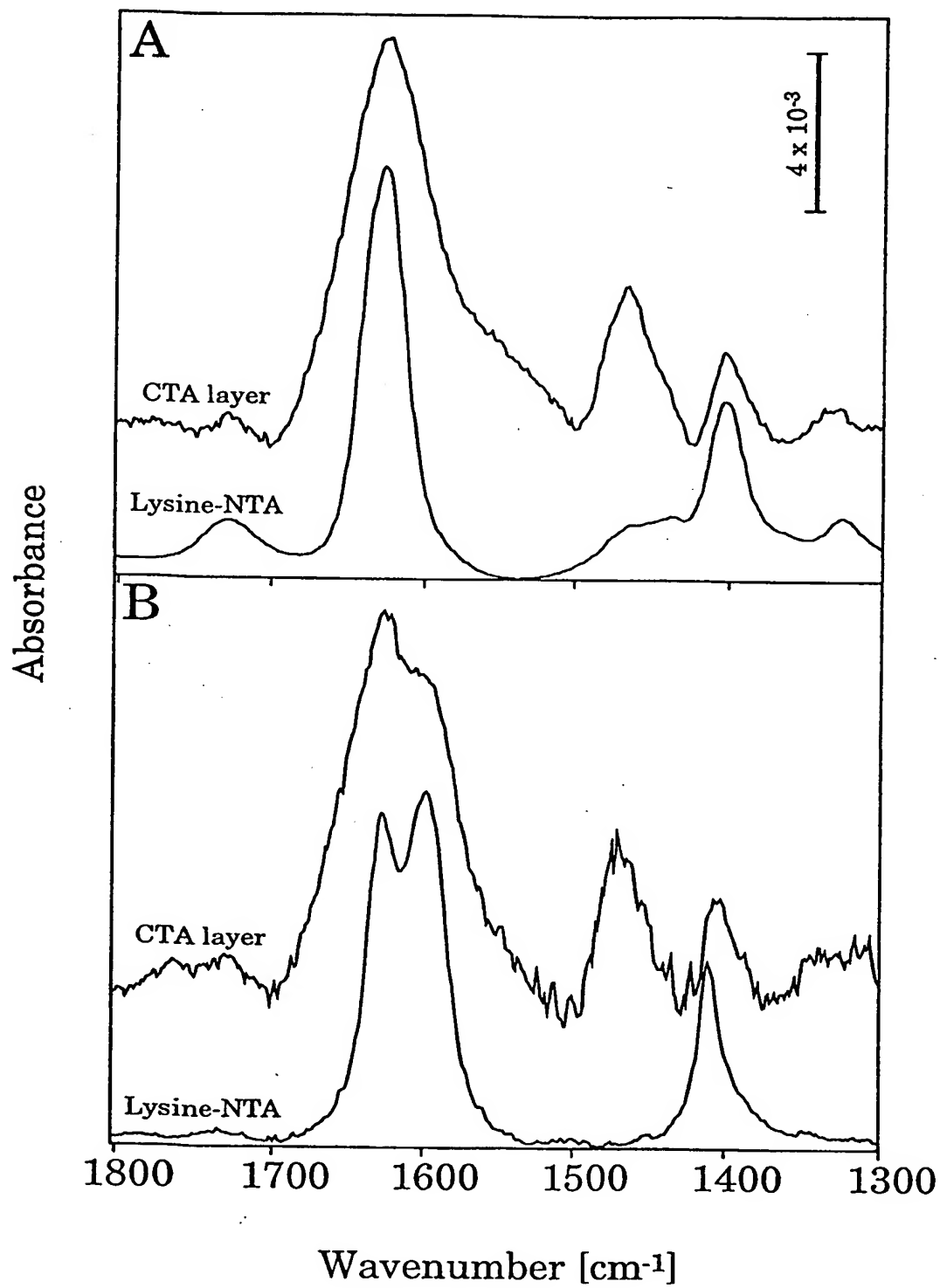


Figure 2

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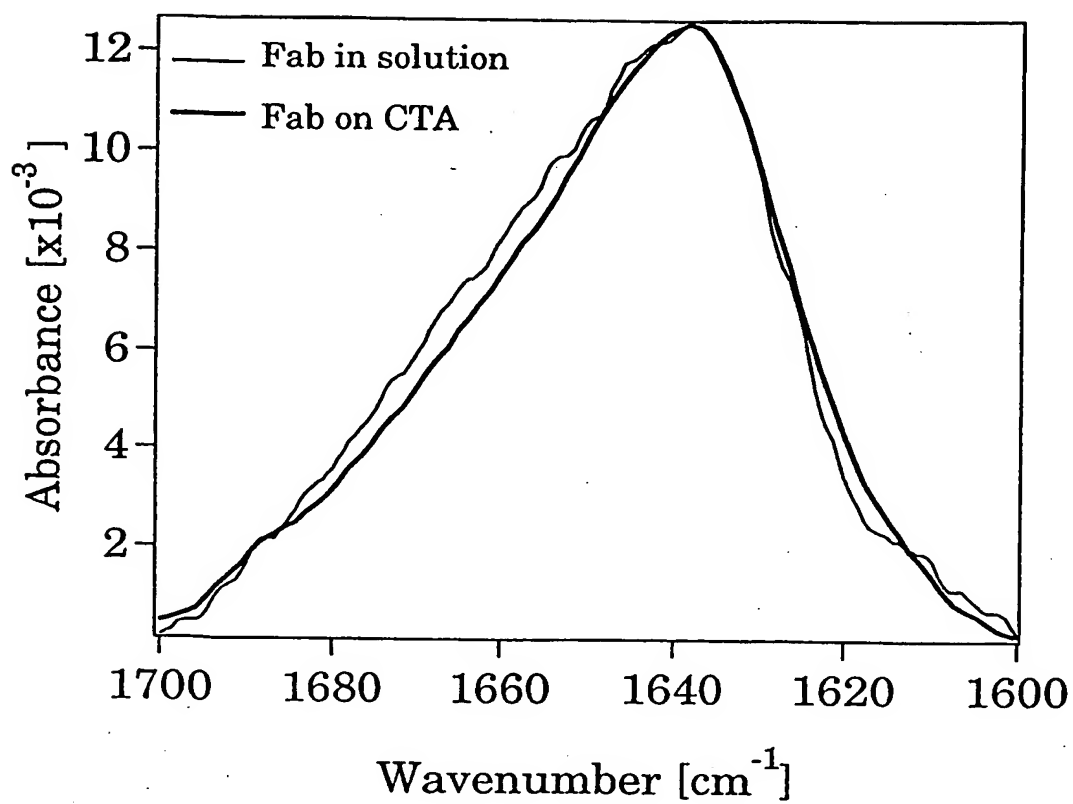


Figure 3

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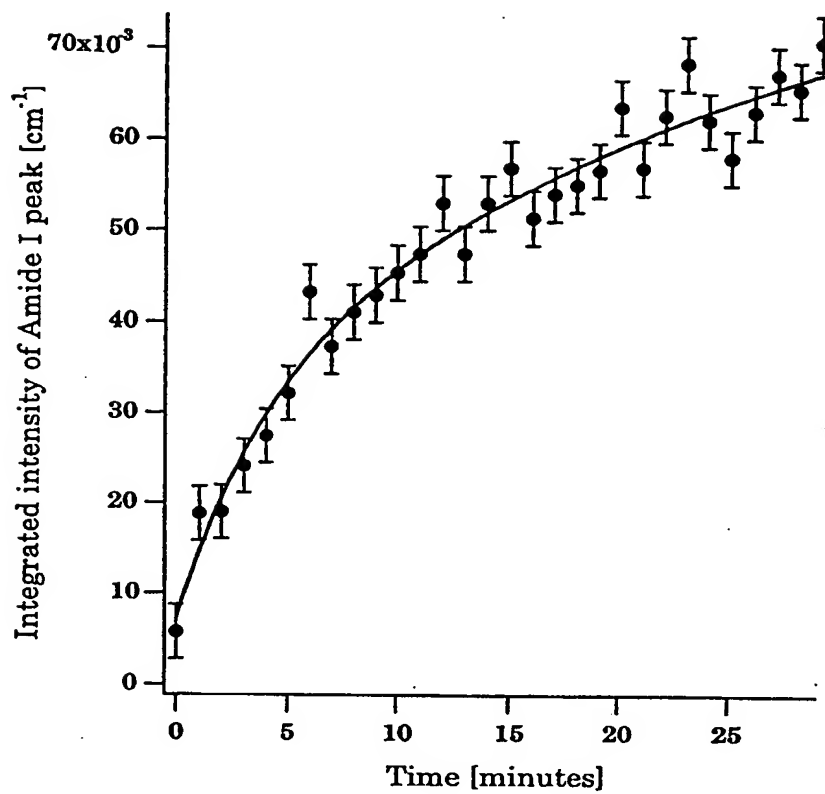


Figure 4.

INTERNATIONAL SEARCH REPORT

International Publication No
PCT/IB 97/00920

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 G01N21/55

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5 629 213 A (KORNGUTH STEVEN E ET AL) 13 May 1997 see column 1, line 62 - column 2, line 12 see column 2, line 52 - column 3, line 2 see column 4, line 16 - line 24; figure 1 ---	1-4, 6-12, 14-17
Y	DE 44 24 336 A (SIGL HUBERT) 18 January 1996 see column 3, line 47 - column 4, line 18 see column 6, line 56 - column 7, line 35; figure 2 ---	1,2,4,6, 9-11, 14-17
Y	US 5 434 411 A (MIYAHARA YUJI ET AL) 18 July 1995 see column 3, line 20 - column 4, line 27; figure 1 ---	1,3,9,12 5,11
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

18 March 1998

Date of mailing of the international search report

8. 04. 98

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INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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